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(54) Title: METHOD AND APPARATUS FOR SIMULTANEOUSLY TESTING A PLURALITY OF COMPOUNDS TO DETECT THEIR ACTIVITY

(57) Abstract

Described is a method of simultaneously testing a plurality of compounds for activity comprising the steps of: (a) placing a plurality of the compounds into at least two arrays, each having a plurality of test zones, with multiple compounds in each zone; (b) determining the location of each compound in each test zone; (c) subjecting the array to a testing screen; and (d) ascertaining those compounds that had a positive response to the testing screen. Also described is an apparatus for performing simultaneous testing of a plurality of compounds in a plurality of arrays containing the compounds to be tested for their activity.

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METHOD AND APPARATUS FOR SIMULTANEOUSLY TESTING A PLURALITY OF COMPOUNDS TO DETECT THEIR ACTIVITY

Technical Field

5   The invention pertains to the field of testing of compounds to ascertain their activity. The invention is particularly concerned with the laboratory testing of large quantities of compounds in a rapid manner.

Background Art

10   Many organizations, in particular pharmaceutical organizations, over many years have synthesized a number of compounds for various research projects. Accordingly therefore, a large collection of compounds have been collected which includes a database of their
15   structures and chemical properties. Frequently, these compounds are screened for biological assays to ascertain the activity of a compound with respect to the assay. A previous method of delivering the compounds involved selecting small numbers of compounds and
20   weighing out the individual samples which were then sent for a single screen. With the advent of automated screening technology, the delivery method became inadequate. A single screen could test hundreds of compounds in a day, while each technician dispensing samples could
25   only deliver a few dozen in a day.

          New equipment became available to speed up the testing techniques. One technique was to manually dispense estimated amounts of individual compounds into small tubes that fit into a 96-well plate format. While

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automated equipment could dissolve the samples and mix them into the reaction wells, they still required a very substantial period of time to go through a screening process. The problem still remained as to how to have the substantial numbers of compounds to be screened for various assays. Preparation of the testing plates and the testing of the compounds per se was extremely labor intensive. It had previously been estimated that it would take almost a year to test approximately 100,000 compounds following this approach.

PCT Publication No. WO93/13423, published July 8, 1993, describes an automated analysis equipment and assay method for detecting cell surface protein and/or cytoplasmic receptor function. The publication teaches an automated measuring apparatus which can decrease substantial worker effort. The publication indicates that for each drug that needed to be screened, the materials were tested one by one, even though an automated apparatus permitted the rapid detection of activity for the compounds tested.

U.S. Patent No. 5,281,540 teaches a test array for performing assays. A semi-automated biological sample analyzer is described for simultaneously performing a plurality of enzyme immunoassays for human IgE class antibodies specific to a panel of preselected allergens in each of a plurality of biological samples. The technique, while having multiple biological samples in a well, uses a coating of an elongated cellulosic body such as a strip of paper which will contact the multiple samples to ascertain which antibodies are specific for the coated allergens and which will then, in turn, bind to the appropriate bands or islands. The bands or islands are then analyzed for the presence of

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labeled antibodies. The technique describes testing
done in a seriatim basis, namely, a number of samples,
one after the other, even though multiple samples are
present in a reaction vessel. The use of antibodies
5   which bind to a specific sample is required for the
system to be effective. The samples may be detected by
use of optical reading capabilities.

Other patents that test multiple compounds in
a seriatim fashion utilizing automated equipment are
10   described in U.S. Patent Nos. 4,039,286 and 4,166,095.

It is an object of the present invention to
simultaneously test a plurality of compounds utilizing
at least two separate arrays of the same collection of
compounds in each array.

15   ## Summary Of The Invention

Described is a method of simultaneously
testing a plurality of compounds for activity comprising
the steps of:
                (a) placing a plurality of the compounds into
20   at least two arrays, each having a plurality of test
zones, with multiple compounds in each zone;
                (b) determining the location of each compound
in each test zone;
                (c) subjecting the array to a testing screen;
25   and
                (d) ascertaining those compounds that had a
positive response to the testing screen.

Also described is an apparatus for simulta-
neously determining the activity of a plurality of
30   compounds comprising:

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(a) a first array of a plurality of test zones, each zone having an ability to contain a plurality of compounds to be tested;

(b) a second array of a plurality of test zones, each zone having an ability to contain a plurality of compounds to be tested;

(c) means for simultaneously testing the compounds in the test zones; and

(d) means for ascertaining which compound in each array has a positive response to a testing screen after it has been determined that a compound has tested positive or negative for the activity.

Brief Description Of The Drawings

FIGURE 1 is a schematic diagram showing the creation of the first array called an X plate utilized in the present invention;

FIGURE 2 is a schematic diagram showing the creation of the second array called a Y plate utilized in the present invention;

FIGURE 3 is a schematic diagram depicting the utilization of Figures 1 and 2 in the mass screening techniques of the present invention using an orthogonal array;

FIGURE 4 is a combination array of a single row A of the X and Y master plates of Figures 1 and 2; and

FIGURE 5 is a master plate activity report showing hypothetical activity for the X and Y plates.

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Description of Preferred Embodiments

The present invention can generally be charac-
terized as a mass screening technique using an orthog-
onal array method (see Figure 3). A simple plate (10)

5   of Figures 1 and 2 utilizes ten columns across the top
row A (of Figure 3). The outermost columns (1 and 12
column numbers) are not used or are used for controls.
Therefore, 80 compounds (8 rows of A-H with ten columns
of 2-11) are placed into a 96-well plate called a

10   "simple plate". The X master plate (12) is made up by
placing an aliquot from each well from simple plate #1
into column 2 of the X master plate (14) and continued
for the remainder of the ten simple plates.

The Y master plate (16) is prepared by taking
15   the same contents of the ten simple plates (10) and
placing the contents of each plate on top of each other
so that the rows and columns of each simple plate fit
within the test wells of the Y master plate (the rows
and columns are the same for simple plate and Y master

20   plate).

A master plate containing 800 compounds (the
X Plate 12), such as a 96-well plastic plate, is pre-
pared. A Y-Plate (16), identified as the orthogonal
array master plate, likewise can contain 800 compounds.

25   As can be seen from Figure 3, if there is a compound
that has activity such as hypothetical compound 727, it
is present on the X Plate in well H4. On the Y Plate,
it is found at well H8. Therefore, the activity has to
correspond to a common compound, hypothetical compound

30   727 in this example. The method likewise is equally
applicable for multiple active materials in a well.

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In its most preferred feature, the current
method is to make two plates that contain the same 800
compounds. One plate is the same as described above ("X
Master plate"), and the other is arranged by turning the
5   array of the first plate 90 degrees ("Y Master plate").
A Beckman robot was used to consolidate ten plates with
one compound in each of 80 wells ("Simple Plate") using
columns 2-11 and leaving columns 1 and 12 empty, into
one plate with 10 compounds in each of 80 wells ("X
10   Master plate") using columns 2-11 and leaving columns 1
and 12 empty (see Figure 1). The consolidation begins
by transferring a 50 μl aliquot from column 2 "Simple
Plate" number one into column 2 of "X Master plate"
number one, then transferring a 50 μl aliquot from
15   column 3 "Simple Plate" number one into column 2 of "X
Master plate" number one, etc., ending with transferring
a 50 μl aliquot from column 11 "Simple Plate" number one
into column 2 of "X Master plate" number one. The
result is that an aliquot from each well of "Simple
20   Plate" number one (10) exists in column 2 of "X Master
plate" (12) number one (14). The same process is used
for the remaining "Simple Plates" two through ten, such
that an aliquot from each well of "Simple Plate" number
two exists in column 3 of the "X Master plate", etc.,
25   ending with an aliquot from each well from "Simple
Plate" number 10 existing in column 11 of the "X Master
plate". The nature of the process ensures that every
compound in row A of "Simple Plate" one through ten
exists in row A of "X Master plate" number one, likewise
30   through row H.

A Tomtec Quadra 96-100 (trademark of Tomtec,
Inc. for automated well filling lab equipment) was used
to consolidate each of the ten "Simple Plates" with one
compound in each of 80 wells, into one plate with 10

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compounds in each of 80 wells ("Y Master plate") using
columns 2-11 and leaving columns 1 and 12 empty (Figure
2). The consolidation begins by transferring a 50 μl
aliquot from each of the 80 wells from "Simple Plate"
5   number one into each of the 80 wells of "Y Master plate"
number one, with the well location of the source "Simple
Plate" matching the well location of the receiving "Y
Master plate". This process continues for the remaining
"Simple Plates" two through ten, such that an aliquot
10   from each well of remaining simple plates is dispensed
in the "Y Master plate", with the well location of the
source "Simple Plate" matching the well location of the
receiving "Y Master plate". The nature of the process
ensures that every compound in row A of "Simple Plate"
15   one through ten exists in row A of "Y Master plate"
number one, likewise through row H. This process also
ensures that the same 100 compounds are in row A of the
"X Master plate" and in row A of the "Y Master plate",
likewise through row H.

20   Both the "X Master plate" and the "Y Master
plate" have 10 compounds in each well while each com-
pound appears once on each plate. However, no two
compounds have the same pair of well locations. When
the plates are tested and the results compared, the
25   individual compounds responsible for any activity can be
determined. For convenience of assigning a location for
each well, Applicant has devised an array combining the
X and Y plates as shown in Figure 4 which is an array of
10x10. For example, after testing "X Master plate" #1,
30   well A2 is determined to be active (containing compounds
1 through 10, inclusive) and after testing "Y Master
plate", well A11 is determined to be active (containing
compounds 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100).
Only compound number 10 is common between the two wells

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determined to be active. Therefore, compound number 10 is assumed to be the active compound. Figure 3 shows a similar hypothetical arrangement. If multiple wells are found in one row on one of the two plates and a single well is found on the corresponding row on the other plate, then one can ascertain the active compounds. For example, if, in addition to the active wells stated above, well A3 from the "X Master plate" is determined to be active (containing compounds 11 through 20, inclusive), then both compound number 10 and compound number 20 are common between the two plates. When multiple wells are determined to be active in the same row on both plates, then estimates may be made of the active material. That is, if well A2 and well A11 from both plates were determined to be active, then compounds number 1, 10, 91 and 100 would be derived from the orthogonal array. However, if only compound numbers 1 and 100 were active, the same four wells would show activity; likewise if only compound numbers 10 and 91 were active.

The advantage of this, even given the probability of testing inactive compounds, is that the biological testers can go directly to quantitative screening with individual compounds. There is no need to create plates that contain the individual compounds. The same plate pairs can be sent to multiple screens, without need to create differently arranged plates for each screen.

How to Identify the "Hit"
Compounds From the X Y Plate Results

In this system, each row can be treated as a distinct entity. That is, any compound that is in row

C on a simple plate, will also fall in row C on both the X plate and the Y plate. The same 100 compounds are in row C on both the X plate and the Y plate. There is no overlap between rows. So each master plate set actually
5   carries eight completely separate 100 compound arrays.

Compounds in all types of plates are dissolved in DMSO (dimethyl sulfoxide), which means they can be transferred using pipeting rather than as solids, which makes it much easier to handle the mixing of very small
10   amounts. However, for follow-up screening of the hit compounds, individual samples are weighed out dry from the sample collection. It is to be appreciated that any inert solvent may be used that is compatible with the testing screen. Such solvents include water, dimethyl
15   formamide, N-methylpyrrolidone, glycols such as diethyl-ene glycol, and the alkyl ether derivatives or the lower alkyl ester derivatives such as the Cellosolve solvents (trademark of Union Carbide).

A Master Plate Activity Report (Figure 5)
20   shows the hypothetical test results on the X and Y master plates.

On the X plate report (20), in row C, there were two hits, in column 3 and in column 9. Now review the list of plate contents shown below in Table I.

25   Since the columns in the X plate were filled in order from left to right, with aliquots from simple plates in numerical order, we know that all ten com-pounds in well 3C on the X plate came from simple plate 1181, and they were all in row C. This narrows the
30   choice to ten compounds from the content list, marked as "pink".

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On the Y plate, in row C, there were also two hits, in columns 5 and 9. Turn to the list of plate contents again. Since the wells in the Y plate were all filled using aliquots from the same wells in the simple

5   plates, we can scan the list of plate contents for all compounds in well 5C, in any simple plate, marked as "yellow".

Now compare the compounds that were marked. The only compound that was marked "pink" and "yellow",

10   was in simple plate 1181, well 5C. The number of this compound is 108240.

When only one well is checked on each plate, in a given row, then its easy to narrow the hit to one compound. It is a little more complicated when two

15   wells are checked on each plate, in a given row.

Look back at row C on the X plate. There were two boxes checked. Using the same method to locate all the compounds in the X plate's well 3C, the compounds in well 9C are marked as "green". All the compounds in the

20   X plates' column 9 came from simple plate 1187. It is seen that one of these ten compounds is also marked as "yellow", because it was in well 5C in the Y plate.

Now look again at row C on the Y plate. There were two boxes checked there as well. The second box

25   checked was 9C. Using the same method to locate all the compounds in the Y plate's well 5C, all the compounds from 9C have been marked as "blue". Each of the compounds in the Y plate's well 9C was also in well 9C on the simple plates it came from. It is seen that not

30   only is one of these compounds marked as "green", there is also one that is marked as "pink".

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So, there are four compounds that were marked twice. Therefore, four hits need to be followed up on. Each of the twice-marked compounds had one mark because it was in a Y plate hit well, and one mark because it was in an X plate hit well. The number of hits in a given row, is actually the product of the number of hits in that row in the X plate multiplied by the number of hits in that row in the Y plate.

Table I

Plate No.	Col.	Row	Compound # Tested	
1180	2	A	100001	
1180	2	B	100002	
1180	2	C	100003	
1180	2	D	100004	
1180	2	E	100005	
		* * *		
1180	4	G	107917	
1180	4	H	107918	
1180	5	A	107921	
1180	5	B	107923	
1180	5	C	107924	*Yellow*
1180	5	D	107934	
1180	5	E	107935	
		* * *		

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Plate No.	Col.	Row	Compound # Tested	
1180	9	A	108022	
1180	9	B	108031	
1180	9	C	108037	*Blue*
1180	9	D	108050	

* * *

1181	2	A	108145	
1181	2	B	108147	
1181	2	C	108151	*Pink*
1181	2	D	108154	
1181	2	E	108160	

* * *

1181	3	A	108169	
1181	3	B	108171	
1181	3	C	108172	*Pink*
1181	3	D	108174	
1181	3	E	108175	

* * *

1181	4	A	108190	
1181	4	B	108194	
1181	4	C	108197	*Pink*
1181	4	D	108200	
1181	4	E	108206	

* * *

1181	5	A	108238	
1181	5	B	108239	
1181	5	C	108240	*Pink-Yellow*
1181	5	D	108241	
1181	5	E	108242	

* * *

1181	6	B	108255	
1181	6	C	108259	*Pink*
1181	6	D	108261	
1181	6	E	108268	

* * *

	Plate No.	Col.	Row	Compound # Tested	
	1181	7	A	108290	
	1181	7	B	108295	
5	1181	7	C	108296	*Pink*
	1181	7	D	108302	
	1181	7	E	108306	

* * *

	1181	8	B	108331	
10	1181	8	C	108332	*Pink*
	1181	8	D	108334	
	1181	8	E	108335	

* * *

	1181	9	B	108355	
15	1181	9	C	108357	*Blue-Pink*
	1181	9	D	108366	
	1181	9	E	108379	

* * *

	1181	10	B	108418	
20	1181	10	C	108426	*Pink*
	1181	10	D	108428	
	1181	10	E	108433	

* * *

	1181	11	B	108445	
25	1181	11	C	108446	*Pink*
	1181	11	D	108451	
	1181	11	E	108452	

* * *

	1182	5	A	108569	
30	1182	5	B	108571	
	1182	5	C	108578	*Yellow*
	1182	5	D	108585	

* * *

	1182	9	A	108769	
35	1182	9	B	108771	
	1182	9	C	108779	*Blue*
	1182	9	D	108788	

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Plate No.	Col.	Row	Compound # Tested	

* * *

1183	5	A	109116	
1183	5	B	109125	
1183	5	C	109126	*Yellow*
1183	5	D	109132	

* * *

1183	9	A	109271	
1183	9	B	109272	
1183	9	C	109277	*Blue*
1183	9	D	109278	

* * *

1184	5	A	109509	
1184	5	B	109510	
1184	5	C	109511	*Yellow*
1184	5	D	109513	

* * *

1184	9	A	109659	
1184	9	B	109666	
1184	9	C	109667	*Blue*
1184	9	D	109672	

* * *

1185	5	A	109933	
1185	5	B	109940	
1185	5	C	109941	*Yellow*
1185	5	D	109942	

* * *

1185	9	A	110082	
1185	9	B	110089	
1185	9	C	110096	*Blue*
1185	9	D	110097	

* * *

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Plate No.	Col.	Row	Compound # Tested	
1186	5	A	110270	
1186	5	B	110272	
1186	5	C	110275	*Yellow*
1186	5	D	110276	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1186	9	A	110420	
1186	9	B	110421	
1186	9	C	110422	*Blue*
1186	9	D	110423	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1187	2	A	110525	
1187	2	B	110527	
1187	2	C	110529	*Green*
1187	2	D	110538	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1187	3	A	110567	
1187	3	B	110568	
1187	3	C	110582	*Green*
1187	3	D	110584	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1187	4	A	110595	
1187	4	B	110596	
1187	4	C	110598	*Green*
1187	4	D	110604	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1187	5	A	110612	
1187	5	B	110614	
1187	5	C	110616	*Yellow-Green*
1187	5	D	110617	

<div align="center">* * *</div>

Plate No.	Col.	Row	Compound # Tested	
1187	6	A	110639	
1187	6	B	110644	
1187	6	C	110650	*Green*
1187	6	D	110652	

<div align="center">* * *</div>

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Plate No.	Col.	Row	Compound # Tested	
1187	7	A	110674	
1187	7	B	110676	
1187	7	C	110684	Green
1187	7	D	110685	

* * *

1187	8	A	110702	
1187	8	B	110707	
1187	8	C	110713	Green
1187	8	D	110716	

* * *

1187	9	A	110747	
1187	9	B	110749	
1187	9	C	110750	Blue-Green
1187	9	D	110760	

* * *

1187	10	A	110793	
1187	10	B	110803	
1187	10	C	110807	Green
1187	10	D	110809	

* * *

1187	11	A	110825	
1187	11	B	110836	
1187	11	C	110838	Green
1187	11	D	110841	

* * *

1188	5	A	110936	
1188	5	B	110940	
1188	5	C	110941	Yellow
1188	5	D	110945	

* * *

1188	9	A	111051	
1188	9	B	111052	
1188	9	C	111053	Blue
1188	9	D	111054	

* * *

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Plate No.	Col.	Row	Compound # Tested
1189	5	A	111249
1189	5	B	111260
1189	5	C	111261 *Yellow*
1189	5	D	111262

* * *

The number of compounds in each test well can vary from 5 to 20, preferably 8-12/well or zone. Ten compounds per well may be an optimum number. If the number of compounds in each well is increased, the number of active wells will increase, and the number of compounds that need to be followed up increases exponentially. However, if three or more arrays (such as a "Z" plate) were used, then more compounds per well could be inserted. The Z plate could be arranged 90° from the "Y" plate for convenience.

Mathematically, the determination of a "hit" can be expressed as follows. Each compound involved in the screening can be uniquely represented by three variables (i,j,k), where k is the simple plate in which the compound is located, and i and j are the row and column, respectively, in which the compound is located in the kth simple plate. Using these variables, a matrix $S_k[i,j]$ representative of the compounds located on the kth simple plate can be formed. Thus, ten matrices can be formed for the embodiment using 800 compounds located on ten simple plates: $S_1[i,j]$, $S_2[i,j]$, ..., $S_{10}[i,j]$. Since eight rows and ten columns are employed on each simple plate in this embodiment, the variable i ranges from 1 to 8 and the variable j ranges from 1 to 10.

The X plate and Y plate are also represented by matrices, namely $X[i,j]$ and $Y[i,j]$. The $X[i,j]$ and

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$Y[i,j]$ matrices are based upon the matrix representation $S_k[i,j]$ of the compounds in the ten simple plates. Specifically, the ten compounds within the ith row and jth column of the X plate are represented by $S_j[i,1]$,

5   $S_j[i,2]$, $S_j[i,3]$, ..., $S_j[i,10]$.   Similarly, the ten compounds within the ith row and jth column of the Y plate are represented by $S_1[i,j]$, $S_2[i,j]$, $S_3[i,j]$, ..., $S_{10}[i,j]$.

As a result, it can be seen that the compound

10   represented by (i,j,k) is located in row i, column k of the X plate, and in row i, column j of the Y plate. This means that a hit due to the compound located in row i, column j of the kth simple plate results in a hit in row i, column k of the X plate, and a hit in row i,

15   column j of the Y plate. Inversely, a hit observed in row i, column k of the X plate and a hit in row i, column j of the Y plate is potentially due to the (i,j,k) compound.

Visually, when a hit is observed at a well in

20   the Y plate, one can conclude that the hit resulted due to an active compound located at the same well location in one of the ten simple plates. A hit in the same row of the X plate can then be used to determine which of the ten simple plates contains the active compound.

25   Specifically, the column number of the hit represents the simple plate which contains the active compound.

Pseudo-code for a procedure for determining the potentially active compounds is as follows.

Do for each row i = 1, 2, ..., 8:

30   Let k1, k2, ... be the column numbers of the hits in row i of X plate;

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Let j1, j2, ... be the column numbers of the hits in row i of the Y plate;

The potentially active compounds are $(i,j1,k1)$, $(i,j1,k2)$, $(i,j2,k1)$, $(i,j2,k2)$, ...

10 simple plates : $S_1[i,j]$, $S_2[i,j]$, ..., $S_{10}[i,j]$

where   i = 1,2,...,8

j = 1,2,...,10

[i,j]:

[1,1]	[1,2]	[1,3]	...	[1,10]
[2,1]	[2,2]	[2,3]	...	[2,10]
[3,1]	[3,2]			.
:	:			:
[8,1]	[8,2]	...		[8,10]

For X plate:

$$x[i,j] = S_j[i,1] + S_j[i,2] + S_j[i,3] + \ldots + S_j[i,10]$$

$$= \sum_{k=1}^{10} S_j[i,k]$$

For Y plate:

$$y[i,j] = S_1[i,j] + S_2[i,j] + S_3[i,j] + \ldots + S_{10}[i,j]$$

$$= \sum_{k=1}^{10} S_j[i,k]$$

$\therefore$ code each compound as (i,j,k) <--> $S_k[i,j]$

<--> row i, column j of plate k

This compound is in : row i, column k of X plate, and row i, column j of Y plate.

=> Inversely, if there is a hit for $x[i,j]$ and a hit for $y[i,j]$, then the compound in $S_k[i,j]$ may be the cause.

The current technique for screening large numbers of compounds is applicable for a variety of screens, most preferably biological screenings. By a

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screen is meant a biological assay that is developed to determine the biological activity of a material.

The collection of materials that are available may be tested against an assay. In this fashion, it can
5   be said that a database of materials can be screened for a particular assay to determine the activity of the portions of the database underneath the assay.

For example, an assay may be followed to determine if a material may be a cholesteryl ester
10 transfer protein inhibitor (CETP). The test is described as follows.

CETP is a 74 kDa plasma glycoprotein responsible for the reciprocal exchange of neutral lipids between circulating lipoproteins. Net alteration in
15 lipoprotein core lipid composition is a complex process. The modifications are influenced by lipoprotein concentration, lipoprotein residence time, and the activities of lecithin:cholesteryl acyl transferase (LCAT), hepatic lipase, lipoprotein lipase, and CETP. In general, in
20 species lacking CETP activity, and humans genetically-deficient in CETP, the equilibrium favors elevation of anti-atherogenic (HDL) and diminution of atherogenic (LDL) lipoproteins. Therefore, plasma CETP inhibition could be an advantageous pharmacological target for the
25 treatment of dyslipidemic patients at risk for coronary heart disease.

Recent studies of a Japanese family with deficiency in plasma CETP have shown that the deficiency was associated with marked elevation of HDL, its associ-
30 ated apolipoproteins (apoA-I, apoE, apoA-IV) and a rarity of coronary artery disease. The defect has been

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identified as a G (guanine) to A (adenine) substitution
in the fourteenth intron of CETP pre-messenger RNA
(ribonucleic acid). This splice donor defect is also
the cause of the deficiency in additional Japanese

5   families identified. In other studies, the deficiency
(both homozygous and heterozygous) has been shown to be
associated with a large proportion of Japanese with
hyperalphalipoproteinemia.

Also, a missense mutation at nucleotide 1506

10   (G for A) has been identified in exon 15 of the CETP
gene, resulting in a substitution of a glycine for
aspartic acid at amino acid 442. The two subjects
heterozygous for the missense mutation had three times
the normal HDL (high density lipoprotein) levels.

15   Overall, these studies suggest that even partial reduc-
tion in CETP levels, as found in heterozygous individu-
als, is associated with elevated HDL. This apparently
benign condition (CETP deficiency) has been coined the
"longevity syndrome".

20   A variety of species, which lack CETP activi-
ty, including mice, rats, and dogs, have HDL as their
major lipoprotein. When fed atherogenic diets, trans-
genic mice expressing human or cynomologus monkey CETP
develop atherogenic lipoprotein profiles, including

25   elevation of apoB containing lipoproteins (VLDL and β-
VLDL) and reduction of HDL. These mice also develop
atherosclerotic lesions. In the transgenic mice, CETP
plasma activity has also been shown to be directly
correlated with apoB and inversely correlated with apoA-

30   I levels. Infusion of antibodies to CETP into rabbits
results in a more favorable lipoprotein profile, includ-
ing elevated HDL cholesterol and particle size. Con-
versely, infusion of CETP into rats results in a less

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favorable lipoprotein profile, including elevation of
VLDL and LDL cholesterol and apoB, and diminution of
apoE-rich HDL cholesterol and HDL size.

Preparation of CETP

5   The d>1.21 g/ml fraction was isolated from
rabbit (Pel-Freez Biologicals, Rogers, AR) or human
plasma and dialyzed against 50 mM Tris, 150 mM NaCl, 2mM
EDTA (ethylene diamine tetracetic acid), pH 7.4 buffer
(1XDB, pH 7.4). Aliquots were stored frozen at -20°C.
10   Chinese Hamster Ovary cells transfected with human
recombinant CETP may be obtained by license agreement
from Columbia University, New York. Media from these
cells grown in 10% fetal bovine serum in Hams F-12 was
used as a source of human CETP without further purifica-
15   tion. The human CETP inhibitory monoclonal antibody TP2
(Mab TP2) may be obtained from Dr. Ross Milne and Yves
Marcel (University of Ottawa Heart Institute). Mab TP2
is also known to inhibit rabbit CETP.

Radioisotopic Whole Plasma CETP Assays

20   Inhibitor screens were performed in 102.5 or
205 μl total volumes in deep 96-well polypropylene
plates (1.2 ml capacity/well) or glass tubes, respec-
tively. Compounds (final concentrations up to 100 μM)
were added in 2.5 or 5.0 μl DMSO and pre-incubated for
25   1 hour at 37°C with previously frozen human plasma (25
or 50 μl). ^{3}H-CL-HDL$_3$ (20,000 or 40,000 dpm) in 75 or
150 μl of 1XDB, pH 8.0 was added and incubated at 37°C.
Wells were harvested periodically up to 24h by the
addition of a 1.0 ml solution (per 102.5 μl incubation)
30   containing 10 mg/ml bovine serum albumin, 1.29 mg/ml
bovine intestinal mucosa heparin (Sigma Chemical Co.,

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St. Louis, MO) in 0.14 M $MnCl_2.4H_2O$ in 1XDB pH 8.0.
Samples were mixed and after 10 minutes centrifuged at
2200 rpm for 30 minutes at 10°C in an IEC PR-6000
centrifuge to precipitate apoB containing lipoproteins.
5   Supernatant aliquots were counted by liquid scintilla-
tion spectroscopy to determine radioactivity remaining
in HDL_3. See JOURNAL OF LIPID RESEARCH, Vol. 34, 1993, pp.
1625-1634, entitled "Use of Fluorescent Cholesteryl
Ester Microemulsions and Cholesteryl Ester Transfer
10   Protein Assays" by C.L. Bisgaier et al.

The invention herein may likewise be used in
the spectrophotometric microtiter-based assay for the
detection of hydroperoxy derivatives of linoleic acid.
See, ANALYTICAL BIOCHEMISTRY, 201, 375-380 (1992) by B.J.
15   Auerbach et al.

An assay for the detection of hydroperoxy
derivatives of linoleic acid formed by the action of 15-
lipoxygenase is described. The assay developed is based
on a method first reported by Ohishi et al (1985) BIOCHEM.
20   INT. 10, 205-211, with some modifications. The assay
described herein takes advantage of the ability of
(9Z,11E)-13-hydroperoxyoctadecadienoic acid (13-HPODE),
the product of the action of 15-lipoxygenase on linoleic
acid, to oxidize N-benzoyl leucomethylene blue to
25   methylene blue in the presence of hemoglobin. The
resultant blue color is stable to light and air and can
be quantified spectrophotometrically at 660 nm. The
linear range of the assay is 1.6 - 3.2 nmol (0.5 - 10
μg) of 13-DPODE. The utility of the assay can be
30   extended to detect other peroxides as well as inhibitors
of 15-lipoxygenase. The assay is a rapid, reliable
method for the detection of lipid hydroperoxide produc-
tion.

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The methods and the materials utilized for
this assay are as follows:

Materials. The following chemicals were
purchased and used as received: linoleic acid (NuCheck
5   Prep), 13(S)-HPODE, NDGA (nordihydroguaiaretic acid),
ETYA (5,8,11,14-Eicosatetraynioc acid), 14,15-methano-
LTA₄ (leukotriene A₄), (Biomol Research Labs), N-benzoyl
leucomethylene blue (Tokyo Kasei Kogyo Co., Ltd.),
dimethylformamide (DMF; Aldrich), sodium cholate, Triton
10   X-100, 30% H_2O_2, 70% t-butyl hydroperoxide, and hemoglo-
bin, bovine (Sigma). Probucol and indomethacin were
prepared at Parke-Davis.

Methylene Blue Method For Peroxide Detection.
The assay is performed in a 96-well microtiter plate.
15   Each well contains 40 μl of substrate solution consist-
ing of 160 μM linoleic acid, 5% ethanol, 0.2% sodium
cholate in PBS without EDTA, inhibitor, if included, and
0.16 U enzyme isolated from phenylhydrazine-treated
rabbit reticulocyte preparations (21) [1 U=1 nmol
20   linoleic acid utilized/min at 4°C] for a total volume of
50 μl. The plate is then incubated at 4°C for 10
minutes followed by the addition of 100 μl of LMB color
reagent consisting of 5 mg LMB dissolved in 8 ml DMF,
which is then added to a 0.05 M potassium phosphate
25   buffer (pH 5) containing 1.4 g Triton X-100 and 5.5 mg
hemoglobin in a total volume of 100 ml. After 5 minutes
at room temperature, the samples can be read at 660 nm
on a microtiter plate reader. Under these conditions,
approximately 20% of the substrate is converted to
30   product.

HPLC (High Pressure Liquid Chromatography)
Method For 13(S)-HPODE Detection. For verification of

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the assay, HPLC analysis of the products was performed
after incubation under the above-described conditions.
The assay is terminated by the addition of an equal
volume HPLC mobile phase (acetonitrile:water:meth-
5   anol:acetic acid, 350:250:150:1). The samples are then
injected onto a C18 column (Perkin-Elmer) with conjugat-
ed dienes monitored at 235 nm and keto-derivatives at
270 nm. A postcolumn chemiluminescence reaction was
utilized to detect hydroperoxy fatty acid derivatives.

10   The invention can likewise be used for deter-
mining the epidermal growth factor receptor kinase
activity.

            The present invention may be used as an assay
for Acetylcholinesterase (AChE) activity. See, for
15   example, the Ellman method (Ashour et al, 1987, ANAL.
BIOCHEM. 166, 353-360).

            The invention may equally be useful as a assay
for mutant reverse transcriptase. This test is a
determination of inhibitors of viral DNA polymerase and
20   reverse transcriptase. See, VIROLOGY, 114, 52 (1981)
entitled "Mechanism of Inhibition of Epstein-Barr Virus
Replication" by A.K. Datta et al.

            The present invention can be used in an RNA
enzyme assay system. The assay is a commercially
25   available assay from Amersham entitled "RNase H(3H)-SPA
Enzyme Assay System".

            The test is equally applicable for looking for
inhibitors of Hepatitis B virus. A typical technique is
called Hepatitis B virus reverse transcriptase assay.

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The assay procedure is a commercially available assay
procedure.

5   The invention is equally applicable to detect
HIV protease enzyme. There is a commercially available
testing identified as Amersham's HIV protease ^{125}I-SPA
assay system.

The invention is equally applicable to deter-
mining the ability of materials for rust-removing
activity, or the ability to dye various textile materi-
10   als, or the ability to clean substrates, or the ability
of material to decompose in the presence of bacteria
such as soiled bacteria and the like.

The invention is equally applicable to deter-
mining a compound's ability to hybridize to a library of
15   genes, or whether particular materials are sensitive to
mammals and the like.

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The invention is equally applicable to deter-
mining sunscreening activity or immune response in
mammals.   Various Elisa (enzyme link immuno sorbant
activity) which enzyme can detect for the presence of a
5   number of biologic materials such as various components
of the blood such as T-cells, B-cells, interleukins, and
the like.

The invention is equally applicable for
determining the presence or absence of a gene which is
10   associated with a particular malady or a gene that is
associated with an absence of a biological response in
mammals. The test could be applicable for determining
the permeability of materials to a membrane such as a
cellular membrane. The test is applicable for determin-
15   ing the activity of a cell to transduce signals over the
ability of different materials to bind two cells or to
bind enzymes or antibodies.   See, CELLULAR AND MOLECULAR
IMMUNOLOGY (2nd Edition) 1994 by A.K. Abbas, pp. 56-60.

It is to be appreciated that a wide variety of
20   a compound's activities could be determined such as:
the activity to be tested of the compounds is
a rust remover activity;
the activity is the ability to dye textile
materials;
25   the activity is to clean substrates;
the activity is the ability to decompose in
the presence soil bacteria;
the activity is the ability of compounds to
hybridize to a library of genes;
30   the activity is the ability to detect sun
screen activity; the sun screens are also detected for
sensitivity to mammals;

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the activity is the ability to induce an immune response in mammals;

the activity is presence of a gene associated with a malady;

the activity is absence of a gene associated with a malady;

the activity is presence of a gene associated with a biological response in mammals;

the activity is the absence of a gene associated with a biological response in mammals;

the activity is permeability of a membrane;

the activity is effecting a signal transduction of a cell;

the activity is ability to bind to a cell;

the activity is ability to bind to an enzyme; and

the activity is ability to bind to an antibody.

The present invention is equally applicable to determining an optimized dosage or weight for active compounds. In other words, the present invention can assist in quantitatively determining active materials and their degree of activity. In this manner, identical or differential amounts of a compound(s) to be tested are placed in various unique well locations.

For a discussion of applicability of the present invention to the simultaneous synthesis of compositions, reference may be made to the concurrently filed patent application entitled "A Method for the Synthesis of Mixtures of Compounds" commonly owned, attorney's case #PD5116, Serial No. _____, hereby incorporated by reference.

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While the forms of the invention herein
disclosed constitute presently preferred embodiments,
many others are possible. It is not intended herein to
mention all of the possible equivalent forms or ramifi-
5   cations of the invention. It is understood that the
terms used herein are merely descriptive rather than
limiting and that various changes may be made without
departing from the spirit or scope of the invention.

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What Is Claimed Is:

    1.   A method of simultaneously testing a
plurality of compounds for activity comprising the steps
of:

5   (a) placing a plurality of the compounds into
at least two arrays, each having a plurality of test
zones, with multiple compounds in each zone;

            (b) determining the location of each compound
in each test zone;

10   (c) subjecting the array to a testing screen;
and

            (d) ascertaining those compounds that had a
positive response to the testing screen.

    2.   The method of claim 1 wherein at least
15 one identical compound to be tested is placed in each
array.

    3.   The method of claim 1 wherein there are
more than two arrays.

    4.   The method of claim 1 wherein there are
20 at least three arrays.

    5.   The method of claim 1 wherein the activi-
ty to be tested of the compounds is a rust remover
activity.

    6. The method of claim 1 wherein the activity
25 is ability to dye textile materials.

    7.   The method of claim 1 wherein the activi-
ty is to clean substrates.

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8.   The method of claim 1 wherein the activi-
ty is the ability to decompose in the presence of soil
bacteria.

5   9.   The method of claim 1 wherein the activi-
ty is the ability of compounds to hybridize to a library
of genes.

10.   The method of claim 1 wherein the zones
contain variations in the concentration of compounds
tested.

10   11.   The method of claim 1 wherein the activi-
ty is ability to detect sun screen activity.

12.   The method of claim 11 wherein the sun
screens are detected for sensitivity to mammals.

13.   The method of claim 1 wherein the activi-
15   ty is the ability to induce an immune response in
mammals.

14.   The method of claim 1 wherein the activi-
ty is presence of a gene associated with a malady.

15.   The method of claim 1 wherein the activi-
20   ty is absence of a gene associated with a malady.

16.   The method of claim 1 wherein the activi-
ty is presence of a gene associated with a biological
response in mammals.

17.   The method of claim 1 wherein the activi-
25   ty is the absence of a gene associated with a biological
response in mammals.

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18.   The method of claim 1 wherein the activity is permeability of a membrane.

19.   The method of claim 1 wherein the activity is effecting a signal transduction of a cell.

5   20.   The method of claim 1 wherein the activity is ability to bind to a cell.

21.   The method of claim 1 wherein the activity is ability to bind to an enzyme.

22.   The method of claim 1 wherein the activity
10   is ability to bind to an antibody.

23.   The method of claim 1 wherein the activity is to determine a cholesteryl ester transfer protein inhibitor.

24.   The method of claim 1 wherein the activity
15   is a kinase activity.

25.   The method of claim 1 wherein the activity is an epidermal growth factor receptor kinase inhibitor.

26.   The method of claim 1 wherein the activity
20   is an acetylcholinesterase activity.

27.   The method of claim 1 wherein the activity is a reverse transcriptase activity.

28.   The method of claim 1 wherein the activity is a ribonucleic acid enzyme assay activity.

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29. The method of claim 1 wherein the activity is a hepatitis B virus inhibitor.

30. The method of claim 1 wherein the activity is a human immunovirus protease enzyme.

5   31. The method of claim 1 wherein the activity is a human immunovirus protease activity.

32. The method of claims 1 or 2 wherein the number of compounds to be tested in each test zone ranges from 5 to 20 per zone.

10   33. The method of claims 1 or 2 wherein the number of compounds to be tested in each test zone ranges from 8 to 12 per zone.

34. An apparatus for simultaneously determining the activity of a plurality of compounds comprising:

15   (a) a first array of a plurality of test zones, each zone having an ability to contain a plurality of compounds to be tested;

(b) a second array of a plurality of test zones, each zone having an ability to contain a plurality of compounds to be tested;

20   (c) means for simultaneously testing the compounds in the test zones; and

(d) means for ascertaining which compound in each array has a positive response to a testing screen

25   after it has been determined that a compound has tested positive or negative for the activity.

35. The apparatus of claim 34 wherein the means for ascertaining the compounds is a computer.

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36. The method of claim 1 further comprising ascertaining a quantitative determination of compound activity by placing in different test zones identical or differential amounts of compounds being tested.
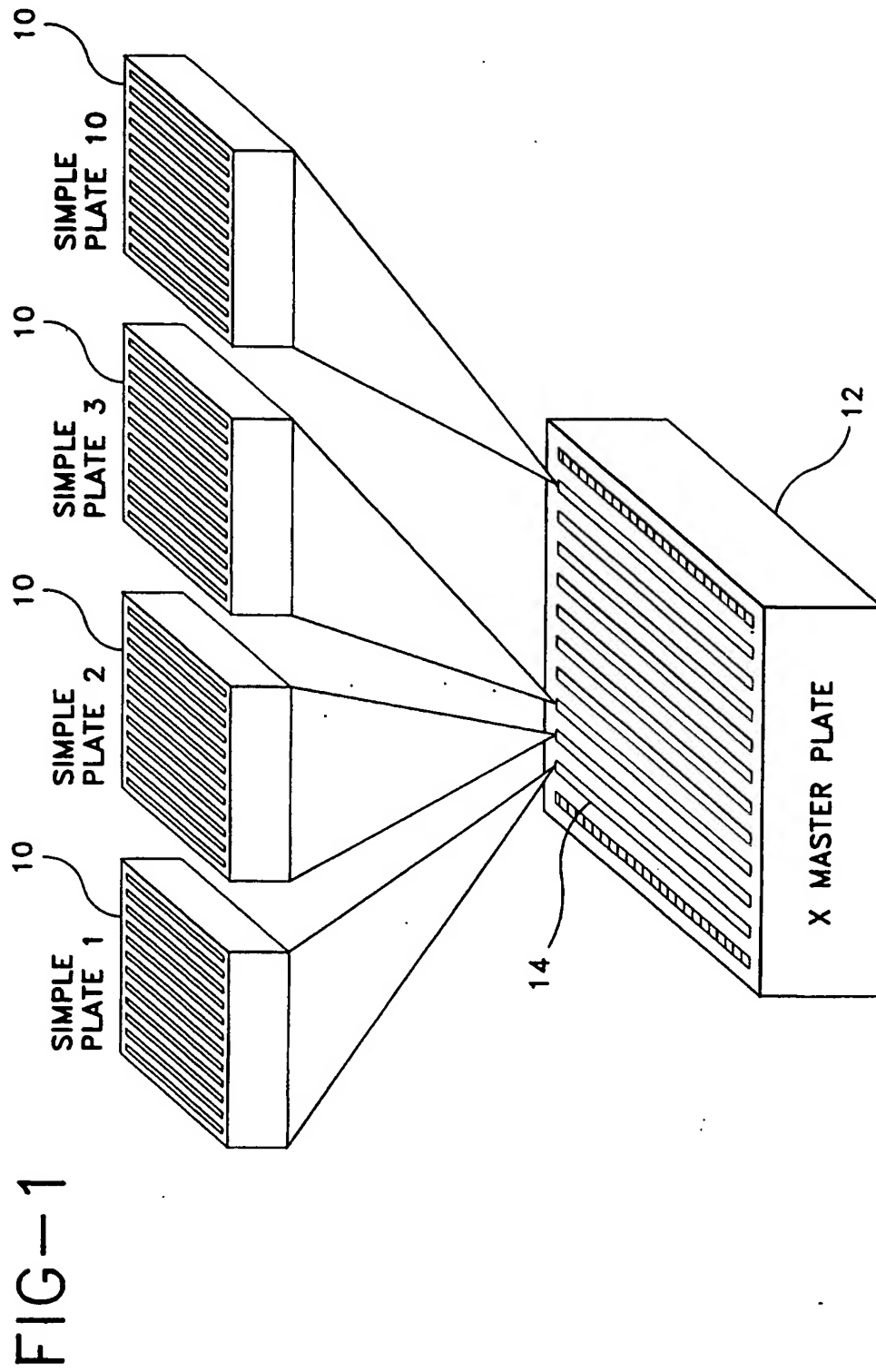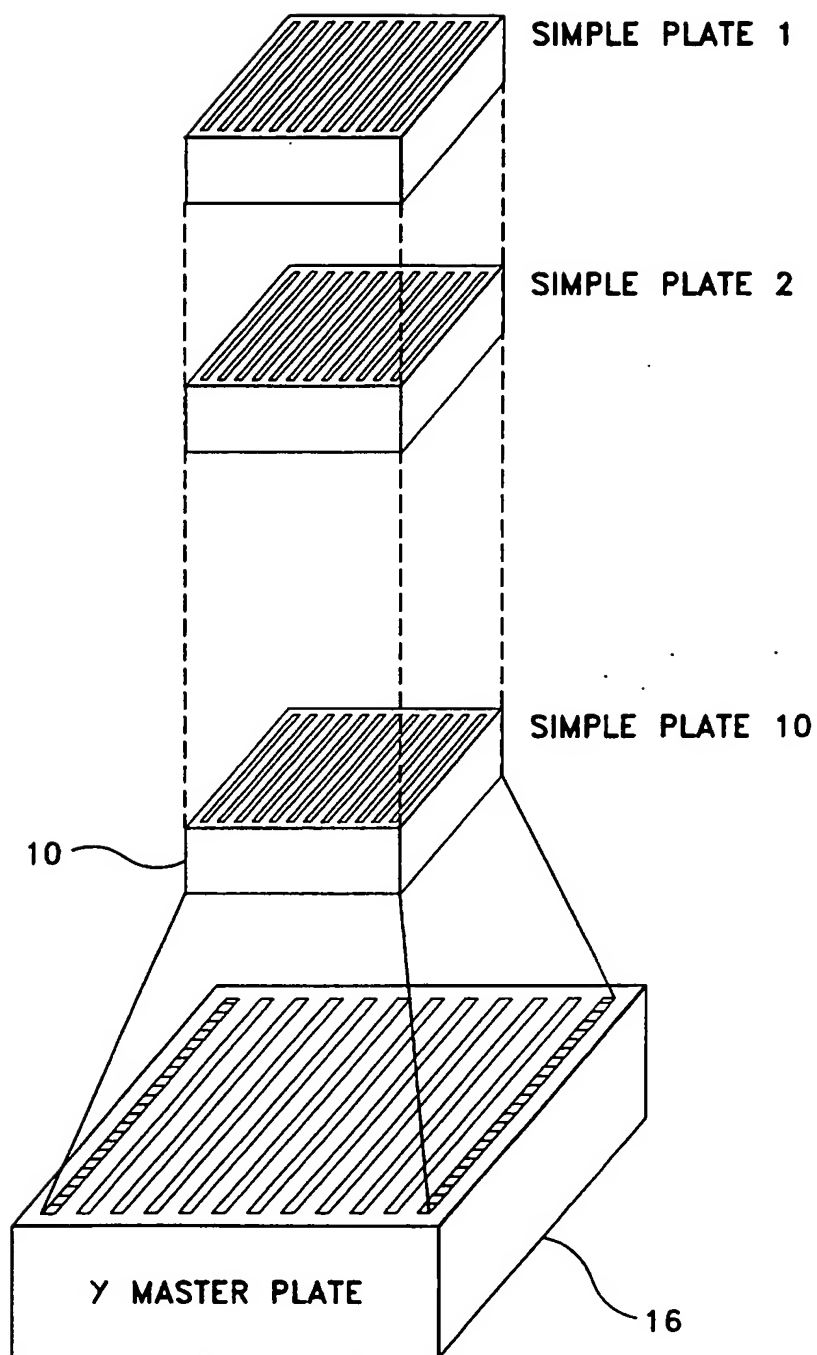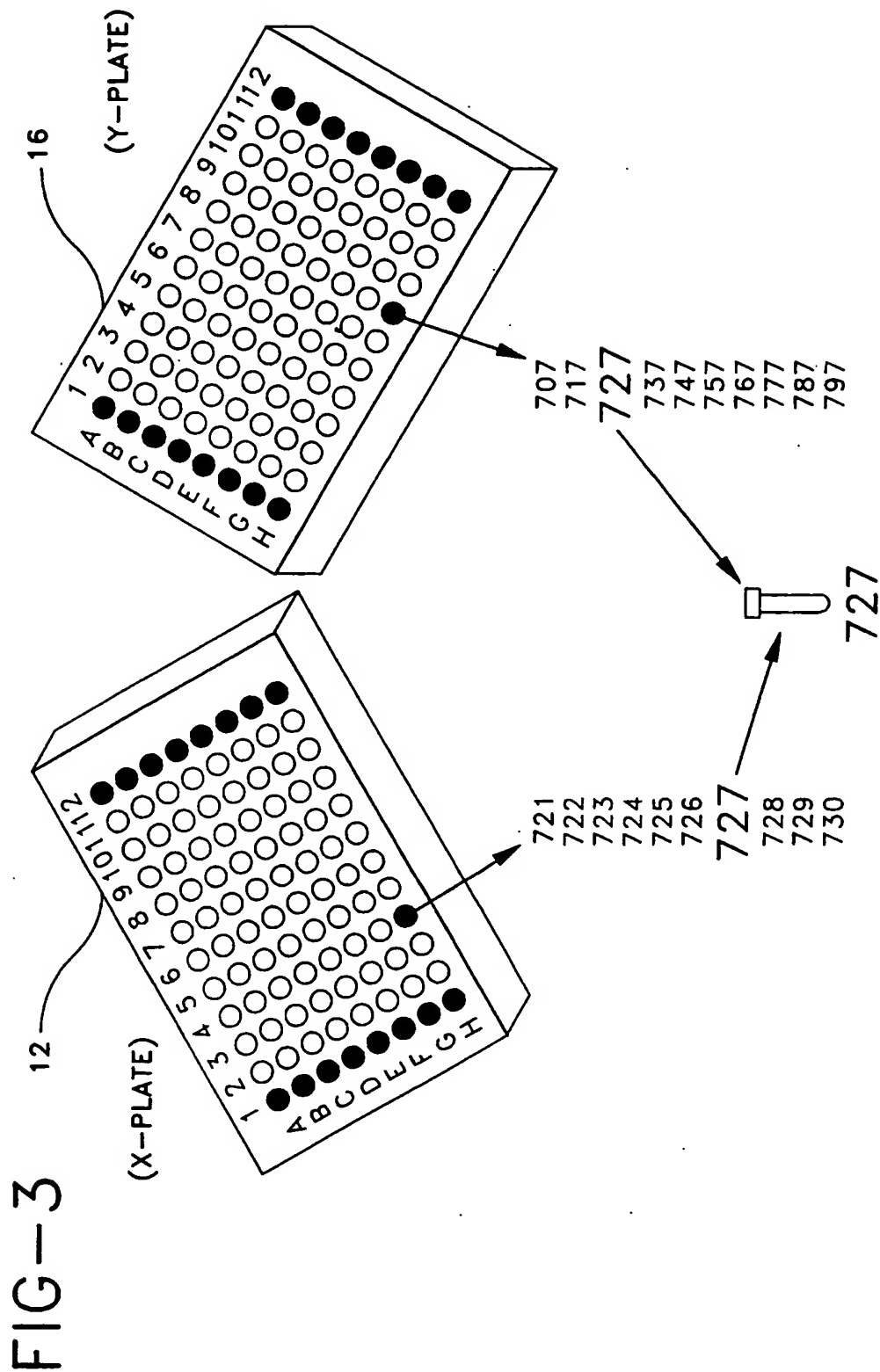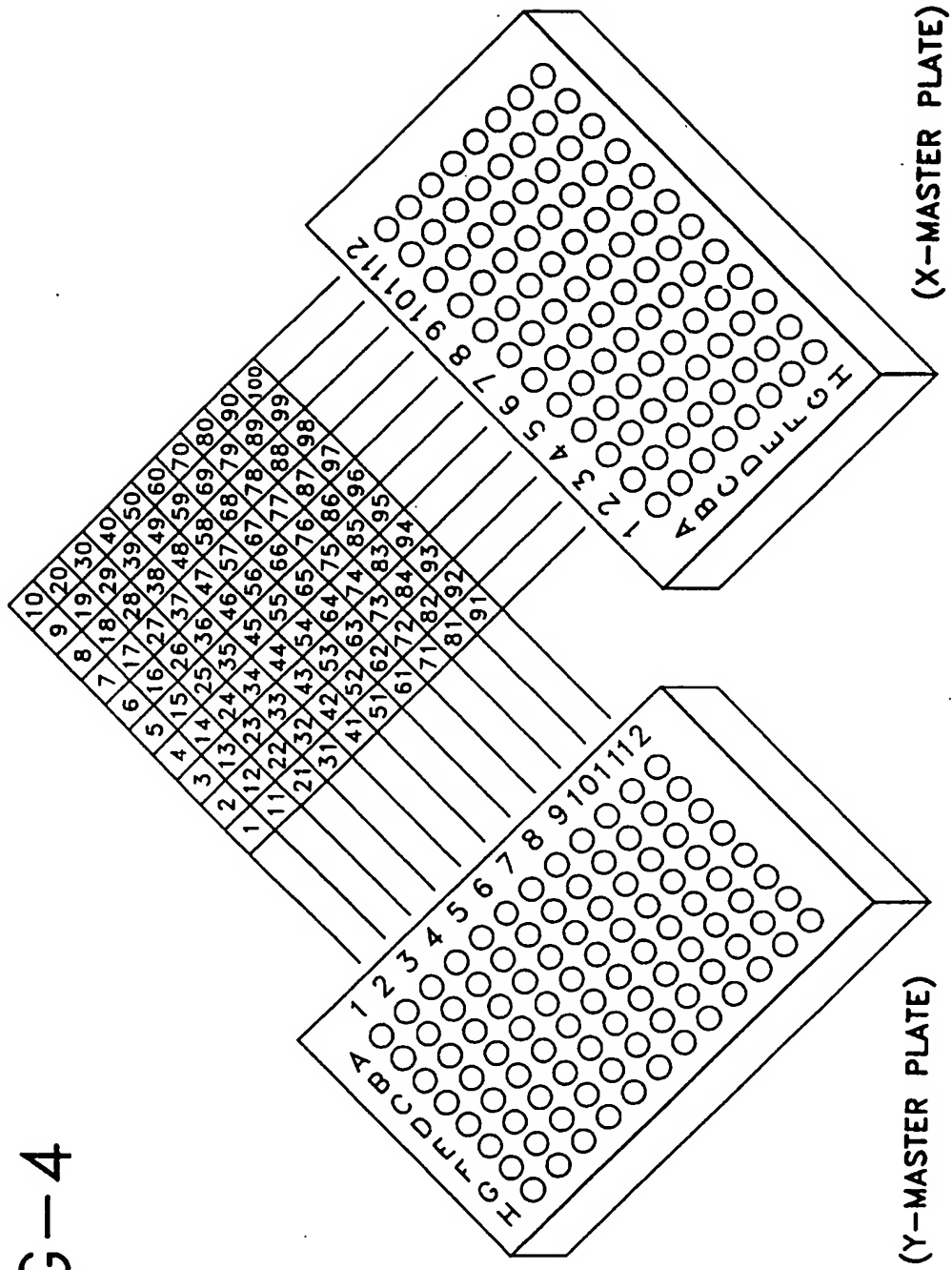
FIG-1

FIG-2
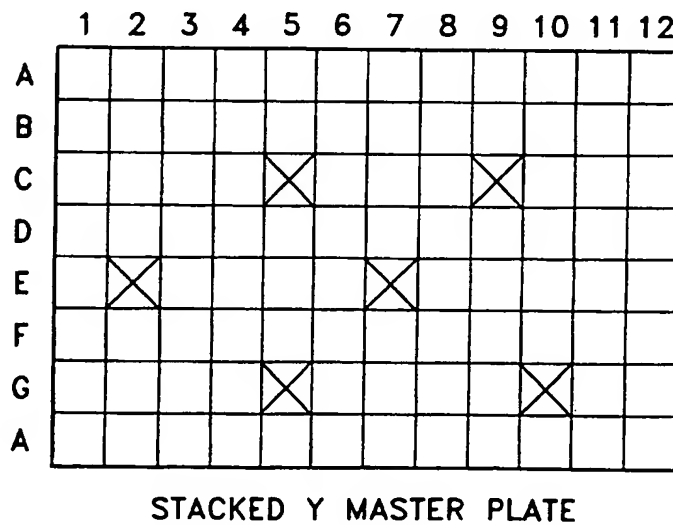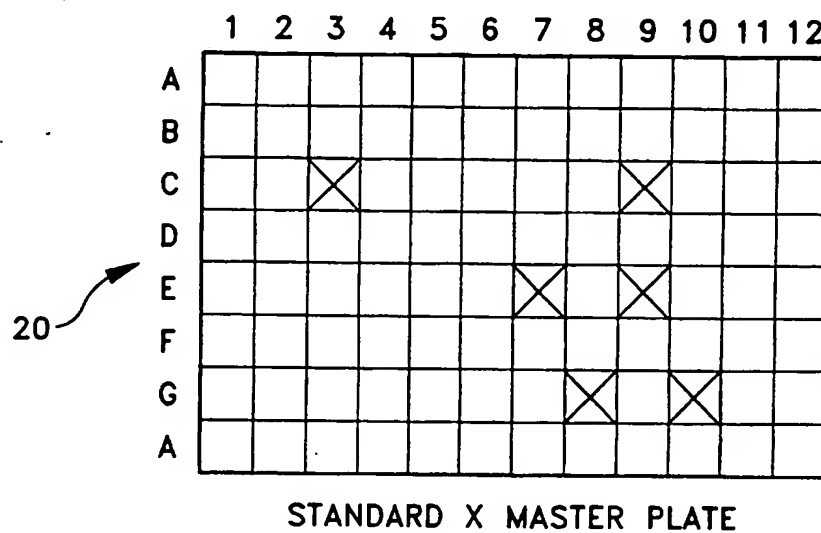

SIMPLE PLATE 1

SIMPLE PLATE 2

SIMPLE PLATE 10

10

Y MASTER PLATE

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FIG-3

FIG-4

FIG-5

STANDARD X MASTER PLATE

STACKED Y MASTER PLATE

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   G01N33/53   G01N33/50   C12Q1/00   G01N35/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   G01N   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 063 810 (CIBA-GEIGY AG) 3 November 1982 see page 3, line 6 - page 4, line 7 see page 18, line 8 - page 23, line 3; figure 1 ---	1-4,34
Y	WO,A,93 13423 (THE SALK INSTITUTE FOR BIOTECHNOLOGY / INDUSTRIAL ASSOCIATES INC) 8 July 1993 cited in the application see the whole document --- -/--	1-36

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 1996	13. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Van Bohemen, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF CLINICAL MICROBIOLOGY, vol. 20, no. 2, 1984, CHICAGO IL USA, pages 255-258, XP000567258 S. DE MARIE ET AL.: "Filter radioimmunoassay, a method for large scale serotyping of Neisseria meningitidis." see page 255, column 2, line 28 - page 256, column 2, line 37 -----	1-36

2

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-63810	03-11-82	AR-A-	231590	28-12-84
		AU-B-	560790	16-04-87
		AU-B-	8306982	04-11-82
		CA-A-	1200761	18-02-86
		GB-A,B	2099578	08-12-82
		JP-A-	58009070	19-01-83
		US-A-	5486452	23-01-96
WO-A-9313423	08-07-93	AU-B-	3416893	28-07-93
		CA-A-	2121506	08-07-93
		EP-A-	0617791	05-10-94
		JP-T-	7505710	22-06-95